Active compound analysis of ethanolic extract of roselle calyces (*Hibiscus sabdariffa* L.)

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ORIGINAL ARTICLE

Abstract

Roselle (*Hibiscus sabdariffa* L.) is a comestible plant known for its fleshy red calyces that are used in making a wide range of foods such as wine, juice, jam, syrup, pudding, cakes, ice cream, and herbal tea. The anti-bacterial, diuretic, anti-oxidant, and anti-mutagenic effects of the roselle calyces are also well known. It is high in vitamins, minerals and bioactive substances such as organic acids, phytosterols, and polyphenols, and because of its extensive pharmacological potential, it has long been used as folk medicine to treat common cold. Gas chromatography-mass spectroscopy (GC-MS) is the best technique to identify the compounds present in the sample by mass spectra data obtained from purely available standards injected under the same conditions. In this study, the GC-MS technique was used to validate the pharmacological potential of *Hibiscus sabdariffa* by identifying the chemicals found in its calyces. The maximum cyanidin-3-glucoside was found to be the highest in PKM (Periyakulam) HS 04 1784.65 mg/100 g in cyanidin-3-glucoside equivalents, total flavonoid content (28.01 mg QE/g), and DPPH (2-diphenyl-1-picrylhydrazyl) activity % (93.17), and in PKM HS 02 total phenolic content (1.29 GA mg/g). The extract was prepared by soaking a dry calyx powder sample in methanol overnight and the ethanolic extract was then analyzed using GC-MS. Flavonoids, tannins, phenols, saponins, alkaloids, glycosides, terpenoids, and steroids were found in the ethanolic extract of *Hibiscus sabdariffa* calyces. The existence of 26 bioactive chemicals was discovered by GC-MS analysis, including phthalic acid, astaxanthin, lutein, lycoxanthin, 3-Pyridinecarboxylic acid, rhodoxanthin, molybdenum, and hexadecenoic acid. The presence of some of these bioactive chemicals has been used to support scientific evidence for the plant's anti-aging, anti-oxidant, anti-hypertensive, and anti-inflammatory capabilities, which constitute valuable preliminary information in pharma industries.

Keywords: GC-MS; roselle; *Hibiscus Sabdariffa*; active compound analysis; pharmacological properties; bioactive chemicals

Introduction

Plants of the Malvaceae family have been found to produce a wide range of structurally diverse fatty acids. They have been employed as medicines in many cultures due to the presence of specific bioactive chemicals for medicinal purposes; they serve as a source for several strong medicament industries (Gopalakrishnan & Udayakumar, 2014). Individual, additive, and synergic activities of phytochemicals are effective in the treatment of various illnesses to health promotion (Patel, 2015). The discovery of active principles in natural sources is the first step in the development of novel medications. Plant extract screening is a novel method for identifying

therapeutically active chemicals in various plant species. Flavonoids, tannins, saponins, alkaloids, and terpenoids are phytochemicals with antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anti-immunomodulatory capabilities (Starlin *et al.*, 2019).

Roselle (*Hibiscus sabdariffa* L.) is a tropical tetraploid (2n=72) plant that belongs to the family Malvaceae. The crop is native to West Africa and was introduced to India as a vegetable in 17th century from Africa. There are over 100 cultivars or seed varieties of *Hibiscus sabdariffa*. The major commercial varieties are those grown in China, Thailand, Mexico, and Africa, principally Sudan, Senegal, and Mali. However, *Hibiscus sabdariffa* can be classified broadly as *Hibiscus sabdariffa var. altissima Wester* and *Hibiscus sabdariffa* var. *sabdariffa.* The former is tall growing unbranched type bearing inedible calyces and is mainly cultivated for the stem fiber. This type is cultivated in some parts of North India. The latter includes the bushy and pigmented type that is cultivated for the edible calyces called roselle. The plant can withstand short periods of drought and can be cultivated in tropics and subtropical areas. The plant is known as Jamaican Sorrel, Karkade, Guinea sorrel, Queensland jelly plant, and Lambadi in some interior parts of North East India. Roselle is also used in traditional medicine. The plant grows up to 4 m tall and has a smooth, cylindrical, typically dark green to red stem. It has a bushy shape, with a dense canopy of dark green leaves that are alternate. The margins of the leaves are toothed. Flowers are borne singly, yellow with a reddish eye, and turn pink at the end of the day. The commercial edible part of the plant is the fleshy calyx sepals surrounding the capsules. The edible fleshy calyces are ready to harvest after 20 days of flowering.

The plant contains flavonoids such as hibiscitrin and hibiscetin. It also contains alkaloids, ß-sitosterol, anthocyanin, citric acid, cyanidin-3-rutinose, delphinidin, galactose, pectin, protocatechuic acid, quercetin, stearic acid, and wax (Shruthi and Ramachandra, 2009). The calyces are rich in acid and pectin. Analysis has shown the presence of crude protein and minerals such as iron, phosphorus, calcium, manganese, magnesium, sodium, and potassium. Mucilage, calcium citrate, ascorbic acid, gossypetin, and hibiscin chloride are also present in the calyces. Several research papers have been published that describe the calyx's anti-hypertensive, hepatoprotective, anti-leukemic, anti-diabetic, anti-bacterial, anti-nociceptive, anti-leukotriene, and anti-oxidant properties, and the most pertinent property of killing tumor cells (Higginbotham *et al.*, 2014). Okore *et al.* (2021) studied compounds present in the leaves of *Hibiscus sabdariffa* through gas chromatography-mass spectroscopy (GC-MS) and revealed the presence of nine organic compounds, namely, cyclohexane carboxylic acid ester, cyclopropane carboxylic acid methyl ester, hexanoic acid-4-octyl ester, hexadeca-2-11-dienoic acid, n-hexadecanoic acid, oleic acid, octadecanoic acid, E-13-docosenoic acid, and E-11-hexadecanal. Olivia *et al.* (2021) investigated the aqueous methanol fraction of *Hibiscus asper* leaves by GC-MS technique to divulge the possible chemical components of which the results revealed the presence of 23 bioactive compounds which include 9,12,15-octadecatrien-1-ol, n-Hexadecanoic acid, octadecatrienoyl acid, and methyl palmitate and phytol. Nandagopalan *et al.* (2015) used the GC-MS technique to determine the possible chemical components in the methanol extract of *Hibiscus tiliaceuss*, which revealed the presence of N, N-Dimethylglycine (83.97%), 3,7,11,15 Tetramethyl-2 hexadecen-1-ol (2.94%), 4HPyran-4-one, and 2,3-dihydro-3,5 dihydroxy-6-methyl (2.69%), providing scientific proof of the plant's anti-bacterial, anti-angiogenic, anti-oxidant, and anti-diabetic qualities. Al-Rekaby (2018) carried out a work to evaluate the bioactive constituents of karkade (*Hibiscus sabdariffa*) where he identified four bioactive constituents: 3,7,11,15-tetramethyl-2-hexadecen-1-ol; 1,1-bicyclohexyl, 2-(2-methylpropyl)-trans; 3-buten-2-one,4-(2-hydroxy-2,6,6-trimethylcyclohexyl); and 1,2-benzenedicarboxylic acid, diisooctyl ester, 3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl). The objective of the study is to characterize the chemical compounds of roselle by GC-MS.

The combined analytical technique of GC-MS is used to uncover and identify chemicals contained in a plant sample. In the phytochemical analysis and chemotaxonomic research of plants containing biologically active components, GC-MS is crucial. With this background, the study aimed to identify the compounds present in the roselle calyx extract.

Materials and Methods

Materials

The seed materials were collected from Singapore and different parts of India, such as Madhya Pradesh, Telengana, Andhra Pradesh, Orissa, and Tamil Nadu, for genotypic evaluation. The source of the seeds chosen for preliminary evaluation is tabulated (Table 1).

Preliminary evaluation

Twenty-five genotypes of roselle were evaluated in Western Block Field at Horticultural College and Research Institute at Periyakulam. Geographically, it is located at 10.126' North latitude, 77.58 East longitude, and an altitude of 426.76 m above mean sea level. The different genotypes were evaluated during 2021–2022 for their yield and total anthocyanin content to choose the best genotype for further GC-MS analysis.

Table 1. Details of the evaluated genotypes.

S. No.	Genotypes	Source	Calyx color	No. of epicalyx segments
01	PKM HS 01	Nanital, Uttarakhand	Dark Red	From 8 to 10
02	PKM HS 02	Hungpung, Manipur	Dark Red	From 8 to 10
03	PKM HS 03	Auroville, Puducherry	Dark Red	From 8 to 10
04	PKM HS 04	Press Colony, Coimbatore	Dark Red	More than 10
05	PKM HS 05	Kadapa, Andhra Pradesh	Red	More than 10
06	PKM HS 06	Guntur, Andhra Pradesh	Reddish Green	From 8 to 10
07	PKM HS 07	Attur, Salem	Light Green	From 8 to 10
08	PKM HS 08	Sandhaipettai, Hosur	Reddish Green	From 8 to 10
09	PKM HS 09	Thirukoilur, Kallakurichi	Red	From 8 to 10
10	PKM HS 10	Chhindwara, Madhya Pradesh	Red	More than 10
11	PKM HS 11	National Gardens, Telengana	Red	From 8 to 10
12	PKM HS 12	Nagakurnool, Telengana	Reddish Green	More than 10
13	PKM HS 13	Ayanavaram, Chennai	Green	From 8 to 10
14	PKM HS 14	Virudhachalam, Cuddalore	Green	From 5 to 7
15	PKM HS 15	Paramathi, Namakkal	Red	From 8 to 10
16	PKM HS 16	Goundampalayam, Erode	Reddish Green	From 8 to 10
17	PKM HS 17	Komarapalayam, Erode	Reddish Green	From 8 to 10
18	PKM HS 18	Thoppapalayam, Erode	Reddish Green	From 8 to 10
19	PKM HS 19	Vadakuthu, Cuddalore	Green	From 5 to 7
20	PKM HS 20	Kamraj Nagar, Dharmapuri	Red	From 8 to 10
21	PKM HS 21	Reddypalem, Andhra Pradesh	Red	From 8 to 10
22	PKM HS 22	Singapore	Deep Red	From 8 to 10
23	PKM HS 23	Balangir, Odisha	Reddish Green	More than 10
24	PKM HS 24	Odisha	Reddish Green	From 8 to 10
25	PKM HS 25	Tiruvannamalai	Light Green	From 8 to 10

(Number of calyx segments and calyx color was categorized based on the descriptors for roselle by The National Centre for Genetic Resources and Biotechnology, Nigeria)

Field trial layout

The field is split into plots and the seeds are sown at a spacing of 1 m × 1 m for evaluation. The evaluation design was randomized block design with each genotype raised in three replications randomly and each plot containing five rows of plants. The periodical readings were taken to assess the growth, yield and, nutritional values of the crop. Roselle contains a pigment that lights up a magnificent red color when used to make culinary products. As the crop is rich in pigments, the best genotype is chosen with the one possessing rich pigments.

Harvesting and drying

The calyces reach maturity 15–20 days after flowering. The calyces are then harvested at their fully matured stage manually and the capsules are removed. The calyces are then oven-dried and powdered for estimation of total anthocyanin content.

Total anthocyanin content

The anthocyanin content of roselle extract was determined using a V-5600 visible spectrophotometer and pH differential spectrophotometry. After dilution with buffers with pH values of 1.0 and 4.5, absorbance was measured at 510 and 700 nm, respectively. The anthocyanin content was calculated according to the following formula:

$$C = \frac{([(A_{510} - A_{700})_{PH1.0} - (A_{510} - A_{700})_{PH4.5}] \times M_W \times L)}{\varepsilon}$$

where C is the anthocyanin concentration (mg/mL), Mw is the anthocyanin"s molecular weight and molar adsorption coefficient, respectively. Because delphinidin-3-O-sambubioside is the most abundant anthocyanin in roselle, its molecular weight (Mw) of 597.1 da was chosen for this study. Sukwattanasinit *et al.* (2007) used a value of 23,800 L/mol•cm in their study and the same was followed.

Total phenolic content

The total phenolic content of roselle was determined spectrophotometrically using Folin–Ciocalteu method modified by Rakesh *et al.* (2021). Dried roselle calyx powder was reconstituted in double distilled water (1 mg/mL). To dilute 100 µL of reconstituted extract, 400 µL of double distilled water was added and 150 L of 1:1 (v/v) diluted Folin–Ciocalteu reagent was added and vortexed. Twenty percent of 500 µL sodium carbonate (Na_2CO_3) was added to the mixture and incubated for 1 h under complete darkness to develop a greenish-blue color. The OD value was measured at 650 nm using a UV-Vis spectrophotometer (Varian Cary® 50 UV-Vis Spectrophotometer). To estimate the phenol content in the extract, 500 µL of ethanol was prepared with all reagents but without roselle extract and gallic acid was used as a standard. Total phenolic content was calculated as mg GA/g.

Determination of DPPH (2-diphenyl-1-picrylhydrazyl) scavenging activity

Rakesh *et al.* (2021) proposed a slight modification to the DPPH (2,2'-Diphenyl-1-Picryl-Hydrazyl) method to estimate the antioxidant activity of roselle calyx powder extract. Since the chemical DPPH is light-sensitive, the experiment was carried out in the dark. In 50% methanol, roselle calyx extract was redissolved. After measuring 50 µL of roselle extract, it was increased to 600 µL by adding 50% methanol. Two hundred microliters of DPPH solution (4 mg DPPH dissolved in 100 mL of methanol) was added to the mixture, vortexed, and kept in the dark for half an hour. A UV-VIS spectrometer was used to measure the change of color and absorbance of the sample solution at 517 nm (Microplate reader, Bio-Rad). As a control, 600 µL methanol with 200 µL DPPH was used without the addition of plant extract. Ascorbic acid was taken as the standard. The free radical scavenging activity (RSA%) was calculated with the formula given below and expressed in percentage.

$$\text{Radical Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{Control}} \times 100$$

where,
$A_{Control}$: Absorbance at 517 nm (Control)
A_{Sample}: Absorbance at 517 nm (Roselle extract)

Total flavonoid content

The total flavonoid content of dried roselle calyx powder was determined using the aluminum chloride method, with a small alteration proposed by Rakesh *et al.* (2021).

One gram of the sample was added with 400 µL double double-distilled water and then added to the 100 µL reconstituted extract and vortexed. Hundred microliters of 10% aluminum chloride solution was added to the sample solution. After that, 100 µL of 1 M sodium acetate was thoroughly mixed with the extract and incubated for 45 min at room temperature in the dark. A UV-Vis spectrophotometer (Varian Cary® 50 UV-Vis Spectrophotometer) was used to measure the development of golden yellow color at 415 nm absorbance. Ethanol 500 µL with all reagents except roselle calyx extract was used as a blank. The extract's total flavonoid content was determined using quercetin as a standard. The total flavonoid content was expressed in mg QE/g.

Sample collection and extraction

The calyces of *Hibiscus sabdariffa* PKM HS 04 were collected from the Western Block field, Department of Vegetable Science at Periyakulam region of Tamil Nadu. The harvested calyces are then dried in a hot air oven at 60°C and pulverized. The powdered dried calyces sample of 10 g was added to 100 mL of ethanol in a beaker and soaked overnight at room temperature. Then, the extract was filtered using Whatman No.41 filter paper.

GC-MS analysis

GC-MS profiling was carried out at the Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore. The instrument Thermo Fisher Scientific GC-MS used a capillary column, TR5MS, 15 m, 0.25 mm i.d., 0.25 µm (PN 76317-3015) in the analysis. The oven temperature was programmed to 70°C for 10 min and then increased to 20°C /min−240°C and held at 240°C. Helium was used as the carrier gas at a flow rate of 1 mL/min.

Components identification

Assessment of the mass spectrum by The National Institute of Standards and Technology's database, which contains more than 62,000 patterns, was used for the GC-MS analysis. The components of the test material's names, molecular weights, and structures were determined by the spectrum of components data in the NIST library.

Results and Discussion

Roselle is a higher plant species of vulgarism. This plant is high in pigments and protocatechuic acid, such as anthocyanins.

The roselle calyx dried samples were tested for their anthocyanin content as mg/100 g unit which was calculated as cyanidin-3-glucosideand it was found to be highest in PKM HS 04 1784.65 mg/100 g in cyanidin-3-glucoside equivalents, total flavonoid content (28.01 mg QE/g) and DPPH activity % (93.17) and in PKM HS 02 total phenolic content (1.29 GA mg/g) was highest than other genotypes (Table 2). The genotype PKM HS 04 was chosen as the best because of its highest anthocyanin content and the extract from the selected best genotype was taken for GC-MS analysis.

The GC-MS analysis results led to the identification of several compounds from the GC fractionations of roselle calyces with the ethanolic extract. These compounds were detected using mass spectrometry in conjunction with GC (Table 1). The compound prediction is based on the National Institute Standards and Technology Database. The primary components and their biological activity found in roselle calyces after GC-MS analysis were tabulated (Table 3). Among them, three components (3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl] tridecyl}-phenyl)-3-phenyl-prop-2-en-1-one, Lycoxanthin, and Astaxanthin) were identified as possessing anti-oxidant property, five (N-[1-(Azetidin-1-carbonyl)-3-oxo-3-phenyl-propyl] carbamic acid, benzyl ester, Lycoxanthin, Lutein, and Hexadecanoic acid) have anti-inflammatory property, five (Astaxanthin, Pregn-4-ene-3,11,20-trione, Octadecanoic acid, 1-[[(1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester, and Withaferin A) have anti-cancerous property, one (Milbemycin b) has anti-parasitic property, and one (3-Pyridinecarboxylic acid) has antimicrobial property. The results revealed the presence of 3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl) phenyl] tridecyl}-phenyl)-3-phenylprop-2-en-1-one (1.14%),

Table 2. Mean values of total anthocyanin content (mg/100 g), total flavonoid content (mg QE/g), DPPH activity (%), and total phenolic content (GA mg/g) in roselle extracts of 25 genotypes.

Genotypes	Total anthocyanin content	Total flavonoid content	DPPH activity (%)	Total phenolic content
PKM HS 01	1406.52	26.54	91.41	0.55
PKM HS 02	1134.81	25.59	90.13	1.29
PKM HS 03	1522.54	25.78	91.08	0.97
PKM HS 04	1784.65	28.01	93.17	0.91
PKM HS 05	482.31	22.85	81.92	0.92
PKM HS 06	693.52	20.64	92.31	0.56
PKM HS 07	3.14	21.93	66.71	0.91
PKM HS 08	154.63	19.87	80.99	0.52
PKM HS 09	316.24	17.70	80.85	0.56
PKM HS 10	549.32	21.20	80.28	0.58
PKM HS 11	634.58	19.89	77.79	0.87
PKM HS 12	548.63	17.07	76.44	0.44
PKM HS 13	2.98	22.29	67.50	0.70
PKM HS 14	1.89	24.78	35.70	0.57
PKM HS 15	309.85	20.67	78.44	0.51
PKM HS 16	120.97	14.33	73.58	0.42
PKM HS 17	148.25	17.33	72.54	0.55
PKM HS 18	148.36	18.06	77.06	0.50
PKM HS 19	1.86	24.10	30.17	0.54
PKM HS 20	275.01	22.95	81.34	0.53
PKM HS 21	306.99	17.26	76.45	0.48
PKM HS 22	1123.05	23.63	85.62	1.08
PKM HS 23	600.98	21.54	73.56	0.76
PKM HS 24	618.09	20.98	82.05	0.81
PKM HS 25	1.71	19.37	25.68	0.57
Mean	509.69	21.38	74.51	0.68
SEd	10.84	0.72	1.40	0.02
CD (0.5)	21.80	1.44	2.80	0.40

Table 3. The results of the compounds identified from GC fractionation.

S. No.	Name	Molecular formula	Molecular weight (g/mol)	Area %	Retention time
1	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl] tridecyl}-phenyl)-3-phenyl prop-2-en-1-one	$C_{43}H_{48}O_4$	628.8	1.14	40.61
2	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.6	6.93	39.5
3	N-[1-(Azetidin-1-carbonyl)-3-oxo-3-phenyl-propyl]carbamic acid, benzyl ester	$C_{21}H_{22}N_2O_4$	366.4	27.23	2.47
4	Lycoxanthin	$C_{40}H_{56}O$	552.9	0.28	14.86
5	Lutein	$C_{40}H_{56}O_2$	568.9	0.28	14.86
6	Astaxanthin	$C_{40}H_{52}O_4$	596.8	0.80	19.42
7	Milbemycin b	$C_{37}H_{53}NO_8$	639.8	0.55	20.44
8	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	1.69	28.02
9	3-Pyridinecarboxylic acid	$C_6H_5NO_2$	123.11	0.51	28.79
10	Pregn-4-ene-3,11,20-trione	$C_{21}H_{28}O_3$	328.4	0.58	32.63
11	Rhodoxanthin	$C_{40}H_{50}O_5$	562.8	3.19	5.75
12	Octadecanoic acid, 1-[[(1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester	$C_{53}H_{102}O_6$	835.4	2.23	7.28
13	Withaferin A	$C_{28}H_{38}O_6$	470.6	0.34	8.38

(Retention time, measure of time taken for a solute to pass through a chromatography column; area %, a reflection of the amount of a specific analyse that is present)

Phthalic acid, di(2-propylpentyl) ester (6.93%), N-[1-(Azetidin-1-carbonyl)-3-oxo-3-phenyl-propyl]carbamic acid, benzyl ester (27.23%), Lycoxanthin (0.28%), Lutein (0.28%), Astaxanthin (0.80%), Milbemycin b (0.55%), Hexadecanoic acid (1.69%), 3-Pyridinecarboxylic acid (0.51%), Pregn-4-ene-3,11,20-trione (0.58%), Rhodoxanthin (3.19%), Octadecanoic acid, 1-[[(1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester (2.23%), and Withaferin A (0.34%) (Figures 1–9). Similarly, upon testing the extracts of hibiscus flower, the results revealed the presence of Ethanimidic acid, ethyl ester (31.43%), Propanal 2,3-dihydroxy (12.58%), Propanamide N-ethyl- (10.69%), Ethylenediamine (6.71%), O-Methylisourea hydrogen sulfate (4.06%), Ethene ethoxy- (3.63%), Hexadecanoic acid, methyl ester (2.99%), 7-Formylbicyclo(4.1.0) heptanes (2.80%), 2- Butanamine (S)- (2.72%), 1,3,5-Triazine-2,4,6-triamine (2.48%), N-Formyl-β-alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), Butanedial (1.65%), 1-Propanol 2-methyl- (1.57%), and Methanecarbothiolic acid (1.08%) (Rassem et al., 2017).

The components identified from the roselle calyx extracts are found to possess several physiological functions and biological properties such as anti-obesity, anti-inflammatory, anti-adiposity, anti-tumor, anti-oxidant, hepaprotective activity, and cardiovascular and cerebrovascular protective effects (Figure 2) (Jeon *et al.*, 2010). Palmitic acid or Hexadecanoic acid is a saturated long-chain fatty acid with a 16-carbon backbone. An anti-inflammatory compound hexadecenoic acid has long been used in the treatment of rheumatism in

Ayurveda. It also works as an emollient that works by smoothening and softening the skin and can be useful in the manufacturing of moisturizing skin care products. It reduces the degree of age-related dark spots, dark pigmentation, and facial pore size in the skin. It is proposed that n-hexadecenoic acid controls swelling in the human body. Another study provides evidence that hexadecenoic acid showed significant cytotoxic effects against cancer cells in humans (Ravi & Krishnan, 2017). The eye is protected from age-related macular degeneration (AMD), the main cause of blindness in the developed world, by the absence of pigments like lutein and astaxanthin. AMD causes central vision blur, damages the macula, and the eyes turn light sensitive, especially in adults. Lutein is a carotenoid member of the xanthophyll family, which has reported anti-inflammatory properties and Astaxanthin is a red pigment that belongs to the carotenoid group of chemicals. Astaxanthin can also be employed as a nutritional supplement for humans, animals, and aquaculture, and has been approved by the US Food and Drug Administration as a food coloring or color additive for specific applications in animal and fish foods. According to several kinds of research, Astaxanthin has anti-tumor, anti-oxidant, cardiovascular, neuroprotective, and anti-apoptosis properties (Donoso *et al.*, 2021; Jia *et al.*, 2017). Rhodoxanthin is a promising water-soluble pigment that imparts blue, red, and purple colors to foods. The natural functional pigment acts as an anti-carcinogenic that inhibits the proliferation of cancer cells (Schex *et al.*, 2020). Osteosarcoma is a type of malignant bone cancer that is common in children and young adults ranging from ages 10 to 20. The octadecanoic acid
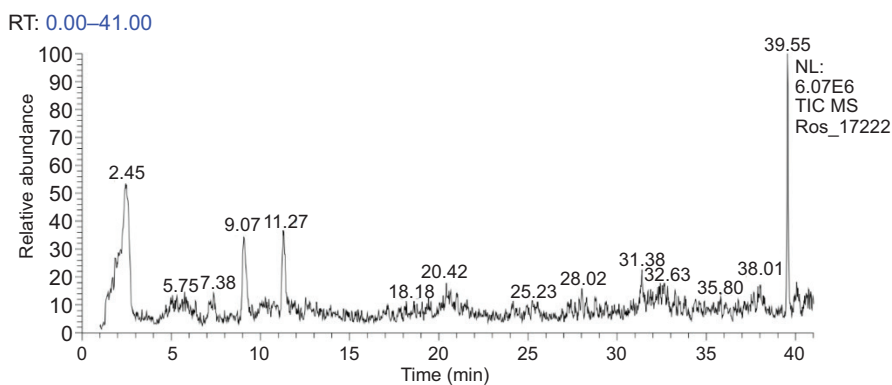
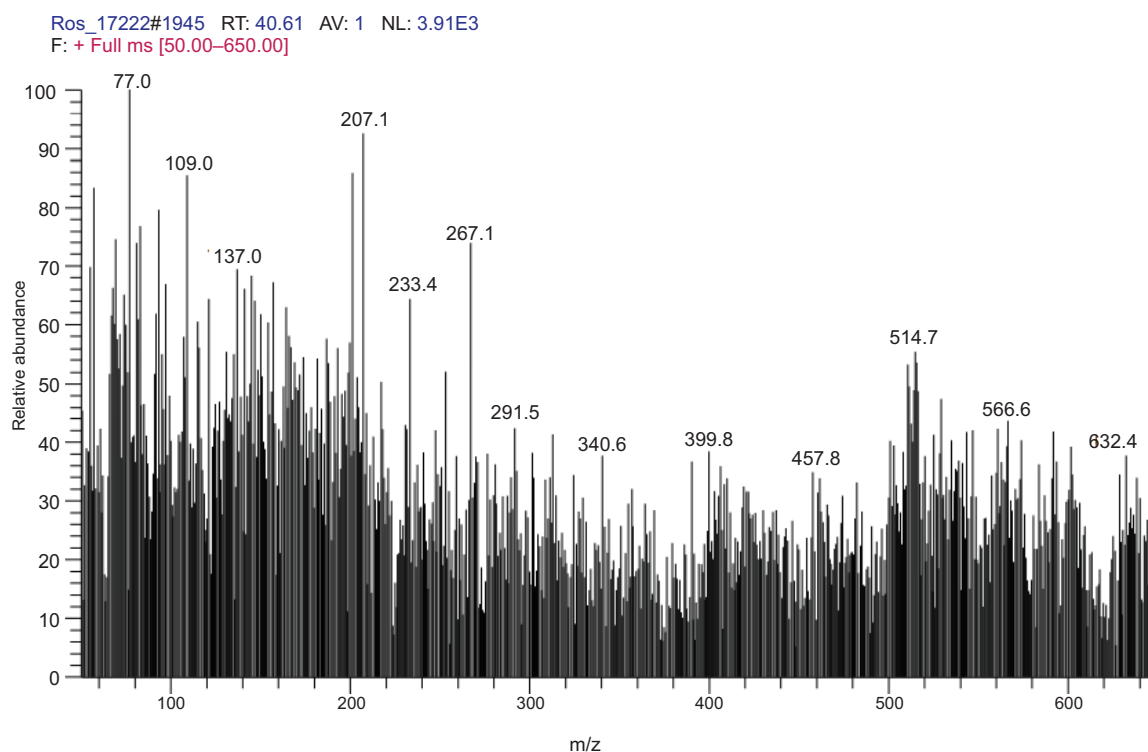**Figure 1.   Chromatogram of ethanolic extract of roselle calyces by GC-MS.**

Figure 2.   Mass spectrum of 3-hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl] tridecyl}-phenyl)-3-phenylprop-2-en-1-one (40.61).

polymer effectively acted as an anti-tumor property aiding the target drug release in the body (Xi *et al.*, 2019).

A pyridine carboxylic acid is a class of organic compounds that are monocarboxylic pyridine derivatives. It comes in three isomers, namely, Picolinic acid (2-pyridine carboxylic acid), Nicotinic acid (3-pyridine carboxylic acid), also known as Niacin, and Isonicotinic acid (4-pyridine carboxylic acid). These compounds are crucial for the effective function of fats and sugars in the body as well as the maintenance of healthy cells. Because of its effects on blood clotting, niacin at high doses may benefit people with heart disease. It may also help to lower triglyceride levels in the blood.

Withaferin A is a steroidal compound, which was the first to be discovered as a member of the withanolide class of ergostane-type products; it was used to treat several illnesses in ancient ayurvedic medicine and recent research has found that the compound has hepaprotective activities and can be used in the treatment of inflammation, liver injury, and liver cancer. Also, Withaferin A exerted cardioprotective, immuno-modulatory, anti-angiogenesis, and cytotoxic anti-proliferative effects against human multiple myeloma cells via apoptosis induction (Xia *et al.*, 2022) (Table 4).

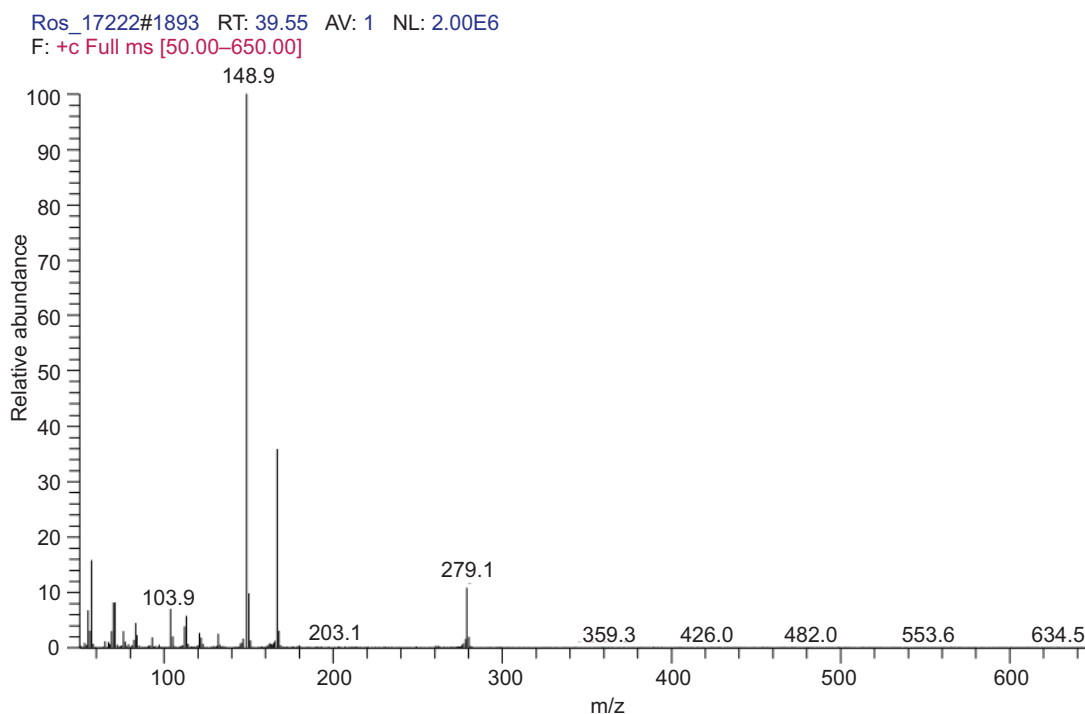As a result, this form of GC-MS analysis is the first step toward understanding the nature of active components

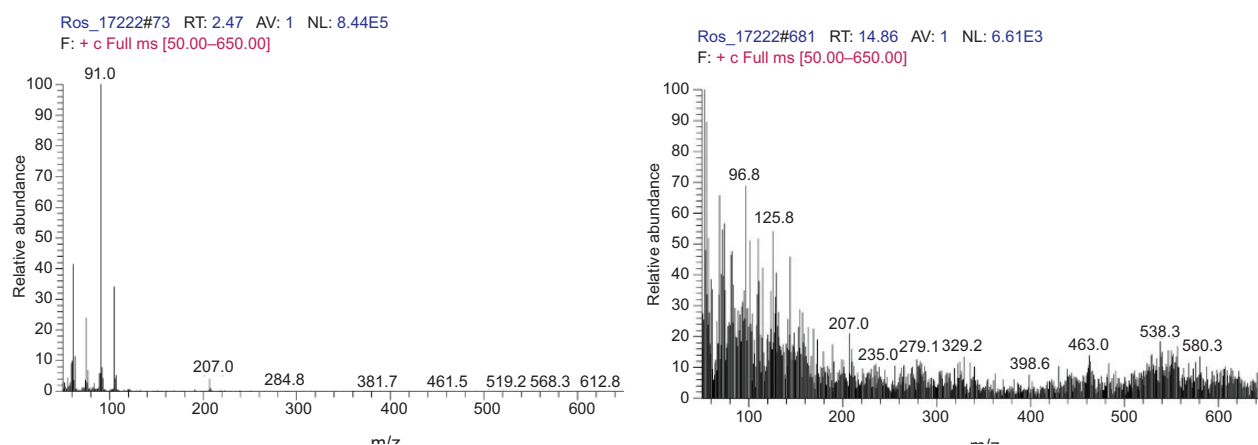**Figure 3.** **Mass Spectrum of phthalic acid and di(2-propylpentyl) ester (39.5).**

Figure 4. **Mass spectrum of lycoxanthin (14.86), n-[1-(Azetidin-1-carbonyl)-3-oxo-3-phenyl-propyl]carbamic acid, and benzyl ester (2.47).**
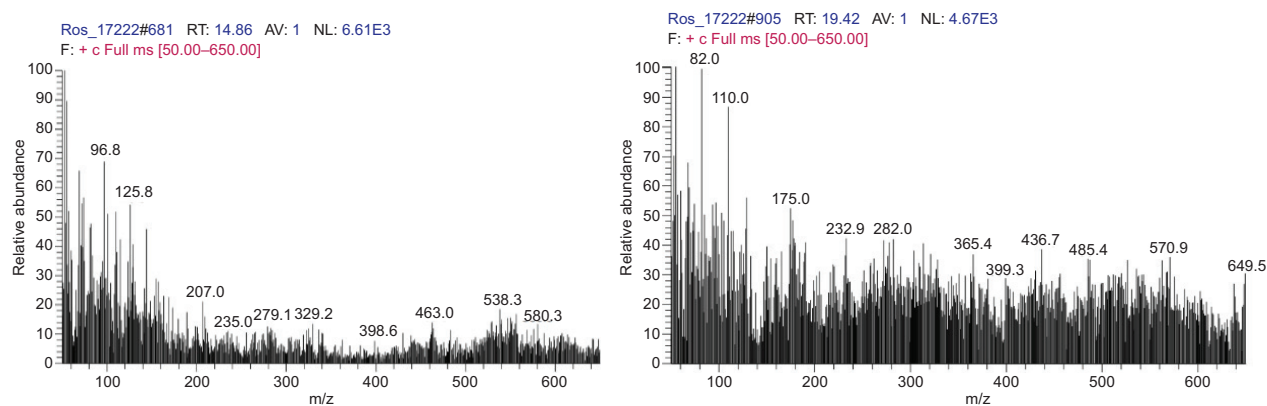

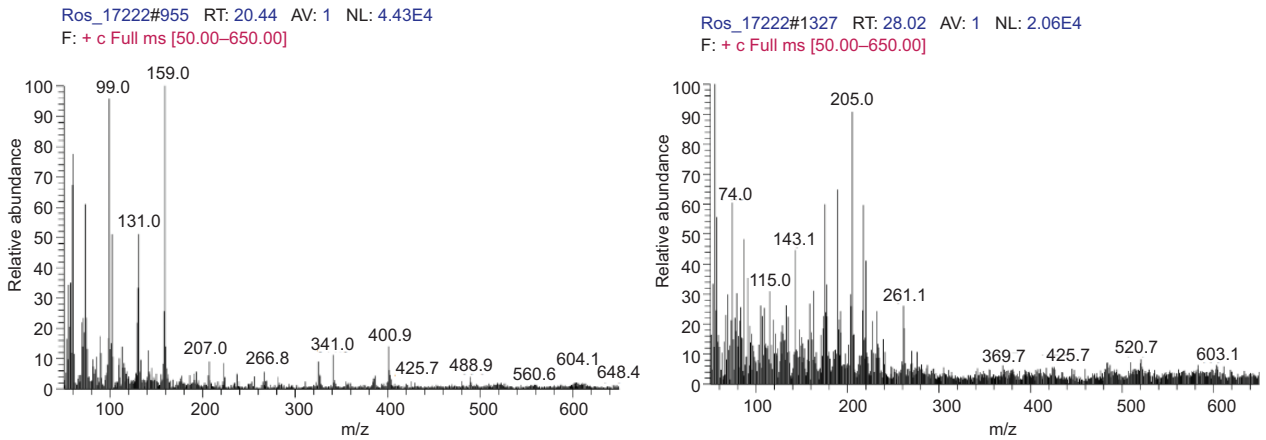**Figure 5.** **Mass spectrum of lutein (14.86) and astaxanthin (19.42).**

Figure 6. Mass spectrum of milbemycin b (20.44) and hexadecanoic acid (28.02).
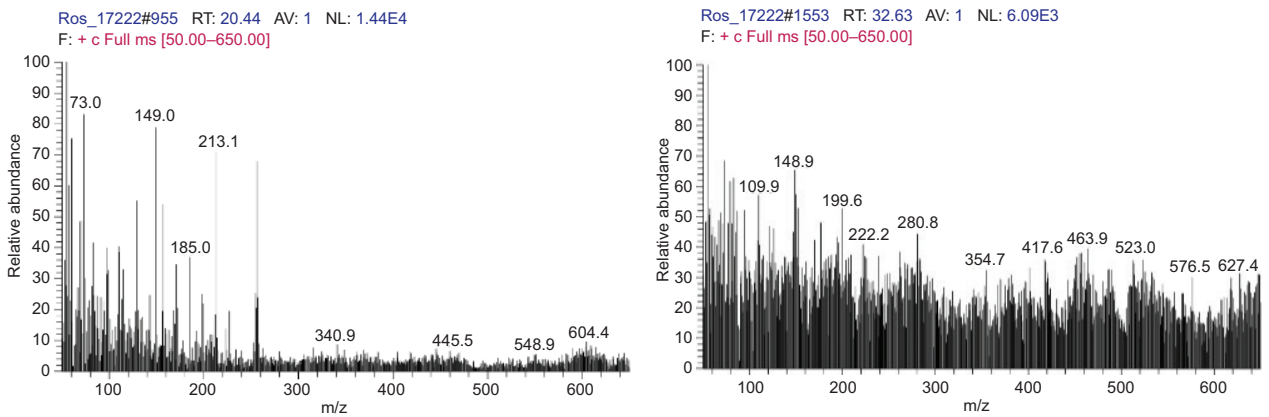
Figure 7. Mass spectrum of 3-pyridinecarboxylic acid (28.79) and pregn-4-ene-3,11,20-trione (32.63).
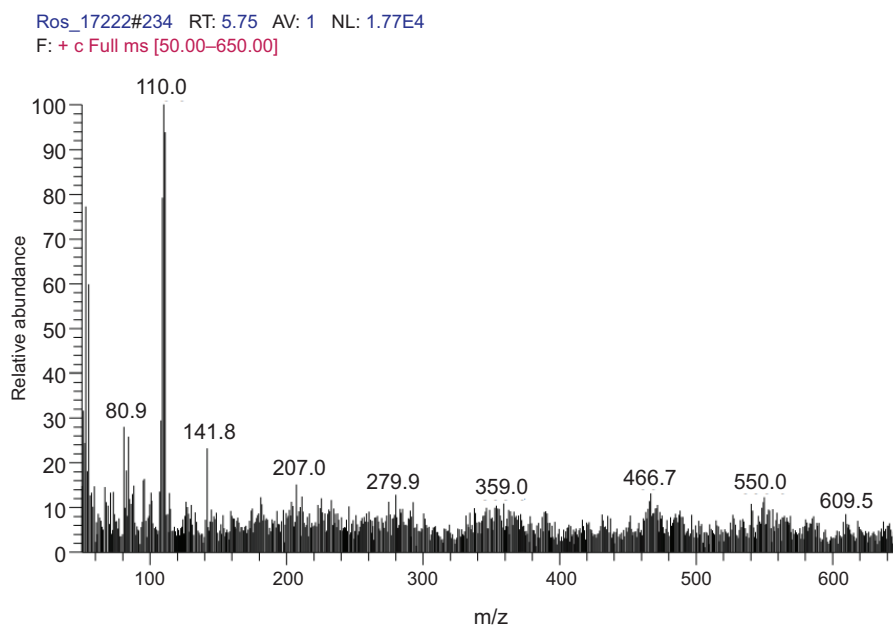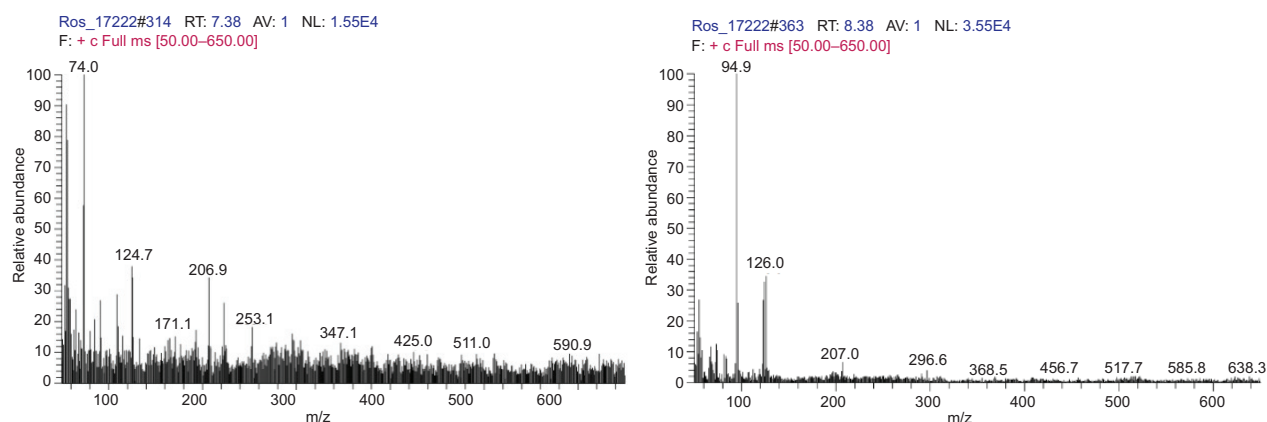
Figure 8. Mass spectrum of rhodoxanthin (5.75).

Figure 9. Mass spectrum of octadecanoic acid, 1-[[(1-oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester (7.28), and Withaferin A (8.38).

Table 4. Roselle calyx components and their biological activity with molecular formula identified by GC-MS.

S. No.	Name	Molecular formula	Activity	References
1	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl) phenyl] tridecyl}-phenyl)-3-phenylprop-2-en-1-one	$C_{43}H_{48}O_4$	Antioxidant	(Al-hadithy, 2020)
2	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	Anti-bacterial, Anti-fungal	(Jasim *et al.*, 2015)
3	N-[1-(Azetidin-1-carbonyl)-3-oxo-3-phenyl-propyl] carbamic acid, benzyl ester	$C_{21}H_{22}N_2O_4$	Anti-inflammatory	(Nuzzi *et al.*, 2016)
4	Lycoxanthin	$C_{40}H_{56}O$	Anti-obesity, Anti-tumor, Anti-diabetes, Anti-oxidant, Anti-inflammatory, and hepatoprotective	(Zhang *et al.*, 2015)
5	Lutein	$C_{40}H_{56}O_2$	Anti-inflammatory prevents age-related macular diseases	(Buscemi *et al.*, 2018)
6	Astaxanthin	$C_{40}H_{52}O_4$	Anti-oxidant, Anti-cancer	(Ambati *et al.*, 2014)
7	Milbemycin b	$C_{37}H_{53}NO_8$	Anti-parasitic	(Scherr *et al.*, 2015)
8	Hexadecanoic acid	$C_{16}H_{32}O_2$	Anti-inflammatory, anti-cancer	(Aparna *et al.*, 2012; Ravi & Krishnan, 2017)
9	3-Pyridinecarboxylic acid	$C_6H_5NO_2$	Anti-microbial	(Mandal *et al.*, 2022)
10	Pregn-4-ene-3,11,20-trione	$C_{21}H_{28}O_3$	Anti-cancer	(Zhang *et al.*, 2015)
11	Rhodoxanthin	$C_{40}H_{50}O_5$	Coloring pigment in foods and beverages	(Schex *et al.*, 2020)
12	Octadecanoic acid, 1-[[(1-oxohexadecyl)oxy] methyl]-1,2-ethanediyl ester	$C_{53}H_{102}O_6$	Drug synthesis-Osteosarcoma	(Xi *et al.*, 2019)
13	Withaferin A	$C_{28}H_{38}O_6$	Anti-cancer, Hepaprotective	(Xia *et al.*, 2022)

in plants and it will be applicable for future research. The pharmacological properties of roselle and its thorough phytochemistry may add to the existing medical knowledge.

Conclusion

The GC-MS results confirm that *Hibiscus sabdariffa* L. may have several significant therapeutic and pharmacological properties for humans. The ethanolic extract of roselle calyces showed the presence of more than 26 compounds. The presence of these compounds has proved the

scientific evidence for their pharmacological potential and several compounds present in roselle were also reported in old works of literature. In this study, compounds identified by GC-MS possess several biological activities such as hepaprotective, anti-oxidant, anti-microbial, anti-fungal, anti-inflammatory, and anti-tumor, which can have great potential in many pharmacological industries. Thus, GC-MS assists us in finding out different kinds of active compounds present in the roselle calyx sample, which helps us comprehend the importance of the crop. Because of the presence of active compounds present in the roselle, pharma researchers and industries are

depending on the raw materials from such a significant crop for drug development and production.

Finally, it would be important to develop new investigations with the compounds identified from the extracts individually, identifying all their potential chemoprotective agents, and pharmacological and nutraceutical properties. In the future, the knowledge of the beneficial properties of *Hibiscus sabdariffa* L. will increase widely throughout the world for its potential in humans.

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